REMARKS

In the Office Action, the Examiner rejected Claims 1-4 under 35 U.S.C. §112, second paragraph as being indefinite because of reference to "a wide variety of dietary antioxidants." The Examiner rejected Claims 1-4 under 35 U.S.C. §103(a) as being unpatentable for obviousness in view of **Chen** and **Coetzee** as applied in the previous Office Action and further in view of a new reference, **Motonaka et al.** The Examiner also rejected Claims 1-4 under 35 U.S.C. §103(a) as being unpatentable over a new reference, **Solinas**, in view of **Chen** or **Christova** and **Coetzee**.

Rejections under 35 U.S.C. §112, second paragraph

Applicant appreciates the Examiner's point concerning "a wide variety of dietary antioxidants." The intent of the term was to emphasize the considerable range of antioxidants detected by the current method. A strength of the method is that an easy to use technique records the presence of a large variety of dietary antioxidants. As demonstrated in the presented data, different antioxidants react at different rates allowing the time course of iodide ion production to demonstrate differences in antioxidant characteristics between ostensibly similar test samples. It is believed that changes in the rate of iodine reduction with time are representative of the presence of different

dietary antioxidants (see especially Table 3 and the discussion related to that table).

As the current method is intended for use with foods and medical samples (e.g., urine), the proper term is most probably "dietary antioxidants" as opposed to the industrial-type rubber antioxidants that were discussed earlier in the prosecution of this case. The general classes of important dietary antioxidants are well recognized and include vitamins (such as Vitamin C) sulfhydryl compounds (such as sulfur containing amino acids) and polyphenolics or tannins (such as flavonoids). While the exact chemical structure or name of each and every antioxidant in a food or a medical sample is often unknown, there is considerable utility in obtaining an overall picture of the status of "dietary antioxidants" in a given sample. Applicant is satisfied that such a term is one for which a person of ordinary skill in the art will have little trouble in appreciating the metes and bounds.

Rejections under 35 U.S.C. §103(a)

Applicant respectfully traverses the rejections under 35 U.S.C. §103(a). Applicant realizes that it is normally not appropriate to overcome an obviousness rejection by arguments directed to individual references. However, Applicant respectfully submits that the reasoning necessary to apply **Solinas**, in particular, goes against the fair teaching of the reference. **Solinas** establishes a

tentative mathematical relationship between peroxide content and natural antioxidants in olive oil. The Examiner somehow is attempting to link this iodometric measurement of oxidants (peroxides) in olive oil with the instant invention, which measures antioxidants. Applicant respectfully suggests that the Examiner somehow has things turned around. This is suggested by the Examiner's statement in the Office Action: "Hence, iodometry derives the measurement of the reducable analyte (in the case of **Solinas**, peroxides) from the amount of iodine consumed in the reaction." Peroxides cannot consume iodine since they are not reductants. The Examiner's attention is drawn to the enclosed explanation of peroxide iodometry with olive oil. In the reaction, the peroxide oxidizes iodide ions and releases iodine, which is measure spectrophotometrically. Oxidation of unsaturated fatty acids (such as those that are found in olive oil) by atmospheric oxygen leads to the formation of peroxides and ultimately to rancidity of the olive oil. Therefore, the higher the quality of the oil, the lower the level of peroxides. Olive oil also contains a number of natural phenolic antioxidants (such as oleuropein) which are partly responsible for the color and flavor of the oil. **Solinas** determined the natural antioxidants by a differential spectrophotometric measurement of phenanthroline-iron complex with or without phosphoric acid. Clearly, **Solinas** teaches that dietary antioxidants are measured by means of phenanthroline. The linkage between **Solinas** and the present invention appears to be that a).

production of iodine is used to measure oxidants (peroxides) and b). the peroxide level is somehow related to dietary antioxidants, which are measured by a phenanthroline method. This is a fairly tenuous relationship. Applicant can see no reasonable way that the teachings of **Solinas** can be combined with any reference to render Applicant's invention obvious. Applicant respectfully requests that the rejections based on a combination with **Solinas** be withdrawn.

w/d Solinas because

In terms of the Examiner's arguments found in paragraph 8 of the Office Action, Applicant agrees that both **Chen** and **Christova** teach that the amount of reductants can be monitored by measuring iodide released from iodine. However, in all fairness **Chen** teaches (see paragraph 3. of translation) that iodine oxidation of ascorbate proceeds only slowly at room temperature. However, in Applicant's invention, such oxidation is rapid. As mentioned in the previous Office Actions, Applicant hypothesizes that this discrepancy is might be a product of buffers or use of iodophors by the Applicant. The Examiner argues that iodophors are rendered obvious by **Coetzee** because that reference teaches that iodophors increase the stability of the reagent. However, that reagent does not teach any effect of iodophors on reaction speeds. Therefore, the artisan trying to make the instant invention by combining the prior art would be faced with the following problem. Following the teaching of **Chen** that ascorbate reacts slowly with iodine at room temperature, the artisan

reference?

An unexpected additional result does not impart patentability when ordinary skill in the art who combined those teachings for other reasons

would not be likely to mix an iodine reagent of the **Chen** type with foods or medical specimens at room temperature because little or no reactivity would be expected. Assuming that **Chen**'s statements are truthful, even if the artisna performed the **Chen** experiment on food or medical samples at room temperature, little or know reactivity would be observed. Applicant's data show that ascorbate is by far the most rapidly reacting reductant. If ascorbate reacts slowly at room temperature (teaching of **Chen**), then the other antioxidants should be even less reactive. The skilled artisan would know that an iodophor (PVP) increases the stability of iodine (**Coetzee**). That reference teaches that PVP-I can be used as a substitute titrant for iodine solutions. The listed advantages are a). stability and b). avoidance of oxidation of acidic iodide. Therefore, if the skilled artisan were making a test of the **Chen** method on room temperature dietary oxidants to see if anything happens, there would be no reason to use PVP. In making a trial experiment, it is not likely to work about reagent stability or potentials for iodide oxidation. Only if the skilled artisan had chosen to use the PVP would the unexpected results of rapid reaction have occurred. The use of PVP to overcome the speed problem pointed out by **Chen** is not based on any teaching of the prior art. Rather the Examiner is making the assumption that because **Coetzee** shows an iodine stability advantage to the use of PVP, then every artisan thereafter would automatically use the PVP reagent. The fact that **Chen** did not try PVP is a

not persuasive Motivation to use more stable form of I_2 is clear

demonstration that this assumption is not valid. Applicant respectfully suggests that the Examiner is using hindsight to add the iodophor to **Chen** to achieve rapid reactions in aqueous solutions. For this reason Applicant respectfully requests that rejections based on the combination of **Chen** and **Coetzee** be withdrawn.

Not only does Applicants invention allow rapid readings at room temperature (something not possible with the cited prior art), Applicant also discovered that different materials show different patterns of rate of reactivity over time (as shown in Table 3). It is believed that different antioxidant components react at different rates so that the prolonged time course of the reaction reveals characteristics about the antioxidants in a sample.

The Examiner's attention is drawn to the enclosed plot of the normalized data taken from table 3. It would be expected that consistent differences in slope are due to different rates of reaction of the antioxidants—or to mixtures of antioxidants that react at different rates. Most probably the changes in slope over time are due to such a mixture. However, flattening of the blueberry curve may represent an exhaustion of the iodine reagent before the antioxidant was entirely oxidized. In any case, the present invention can detect changes in antioxidant reaction over time, which allows one to distinguish antioxidant

characteristics of samples that would appear identical with a single time point reading. This additional aspect is claimed in new claim 6.

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner still finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles telephone number (310) 734-5200 to discuss the steps necessary for placing the application in condition for allowance.

You are hereby authorized to charge any fees due and refund any surplus fees to our Deposit Account No. 50-1796.

Respectfully submitted,

CROSBY, HEAFEY, ROACH & MAY

Date: 30 September 27, 2002 By: Stefan J. Kirchanski

Stefan J. Kirchanski
Registration No. 36,568
Attorney for Applicants

1901 Avenue of the Stars, Suite 700
Los Angeles, CA 90067
Telephone: (310) 734-5200
Facsimile: (310) 734-5299

Attachments:

Web page on olive oil peroxides
Plot of Table 3

Red-lined Claim Copy (Revised Rule 121)

1. (Three Times Amended) A method for determining a composite measure indicative of the presence of [a wide variety of] dietary antioxidants in a liquid sample at room temperature comprising the steps of:

providing a liquid sample containing dietary material or a biological fluid to be tested;

contacting the liquid sample with an aqueous solution of elemental iodine and an iodophor at room temperature to form a mixture; and

measuring a change in a concentration of iodide ions in the mixture at room temperature wherein the change represents the composite measure of the presence of [a wide variety of] dietary antioxidants in the dietary material or the biological fluid.

4. (Three Times Amended) A method for determining a composite measure indicative of the presence of [a wide variety of] dietary [antioxidant] antioxidants in an aqueous liquid sample at room temperature comprising the steps of:

providing an aqueous liquid sample containing dietary material or a biological fluid to be tested;

contacting the sample with an aqueous solution of elemental iodine and polyvinylpyrrolidone at room temperature to form a mixture; and

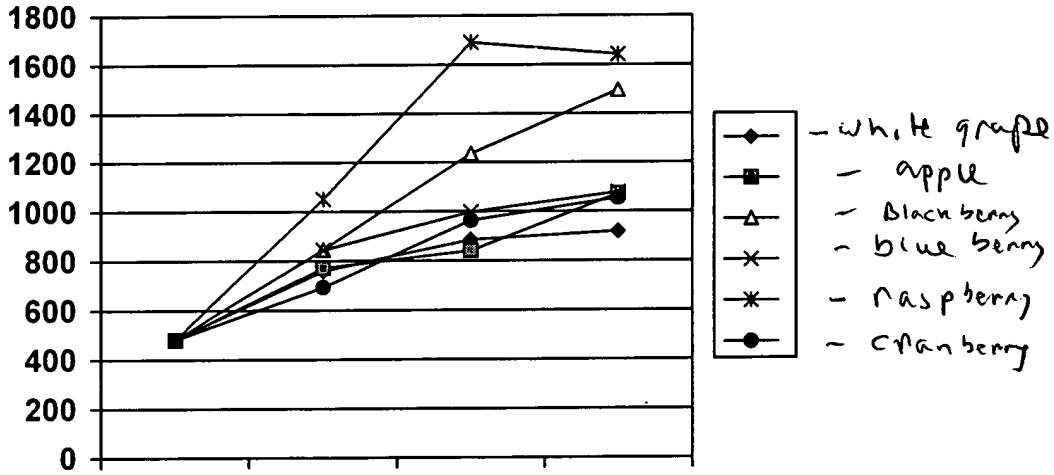
measuring an increase in a concentration of iodide ions in the mixture by means of an iodide selective electrode at room temperature wherein the increase represents the composite measure of the presence [of a wide variety of] dietary antioxidants in the dietary material or the biological fluid.

97	375	501	535
466	756	826	1056
481	846	1236	1496
339	703	856	936
1216	1786	2426	2376
263	475	746	836

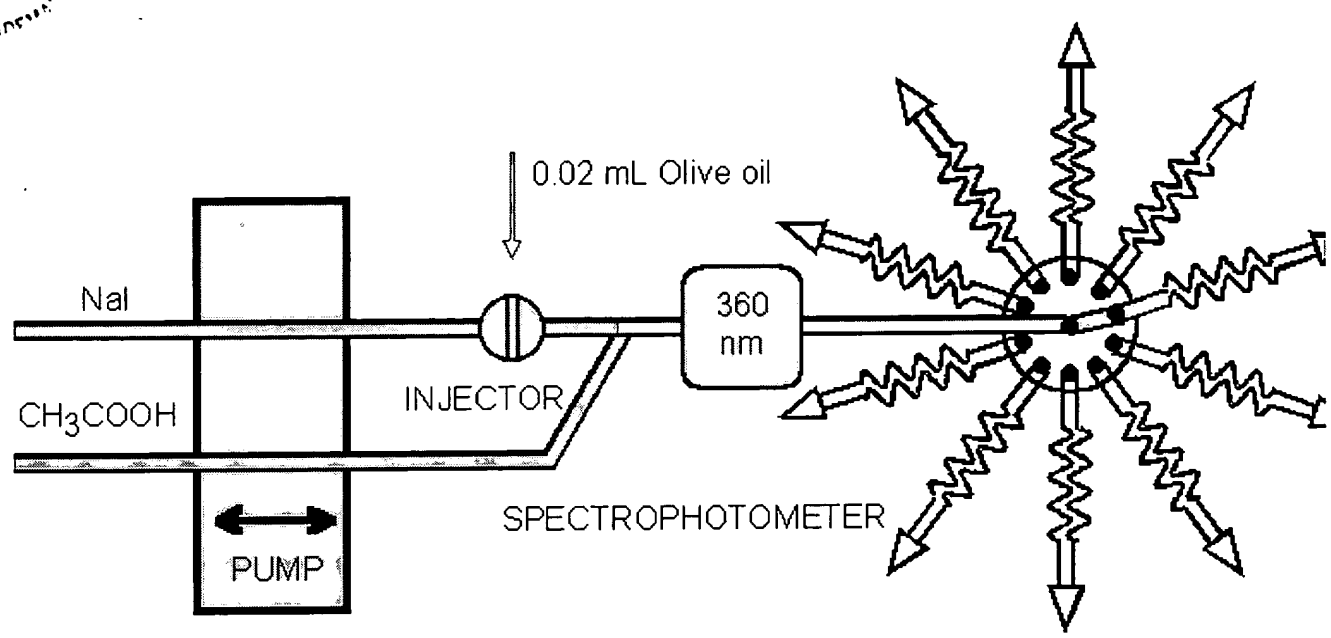
Table 3 data

481	759	885	919
481	771	841	1071
481	846	1236	1496
481	845	998	1078
481	1051	1691	1641
481	693	964	1054

Table 3 data normalized



Automated Olive Oil Peroxide Value Deter by Flow Injection

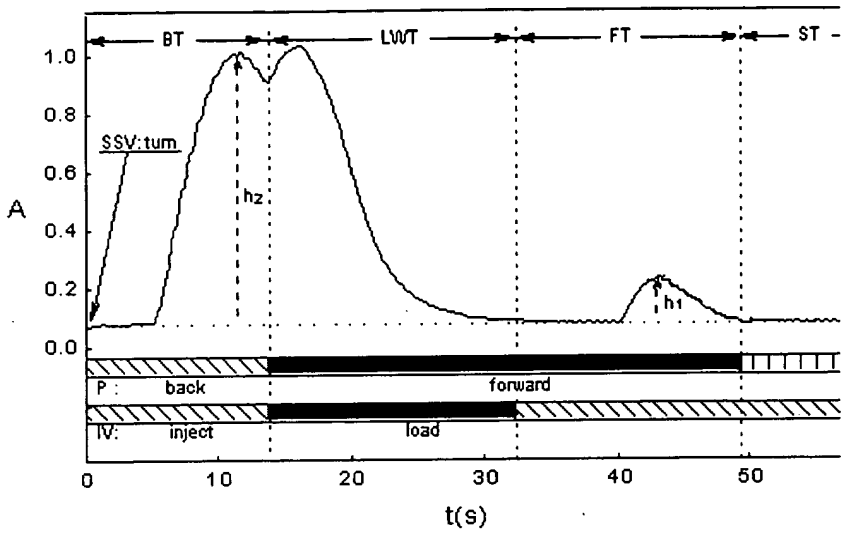


STREAM SELECTION VALVE AND INCUBATION COILS

- Olive oil samples are injected in 1.0% (w/w) NaI and acidified by a 43.2 % (v/v) CH_3COOH stream in n-propanol. The iodine liberated by peroxides is continuously monitored.
- The reaction mixture flows through the spectrophotometer where sample blank values are recorded. Then, it is diverted to the incubation coil selected by the stream selection valve. After loading 7 incubation coils, the pump reverses the flow direction to aspirate incubated samples f measurement. Finally by changing again the flow direction samples are driven to waste. The timing sequence and a calibration run are shown in the following figures.
- In this way, an analysis rate of 100 samples per hour is achieved while each sample is incubated for 5 min. With the proposed analyser design, the drawback of "one sample at a time" is overcome as 7 samples reside simultaneously in the analyser, resulting in high analysis rates.
- In flow injection methods, the *reagent(s) blank* is the baseline signal. However, the *sample blan* value that can be quite high, is not automatically subtracted. By the proposed analyser design th analytical signal is corrected for the sample blank value.
- Results obtained by the proposed method compare well with those obtained by the official meth (0-5.6% relative difference for the analysis of 27 olive oil samples). Click to see other advantag of the method.

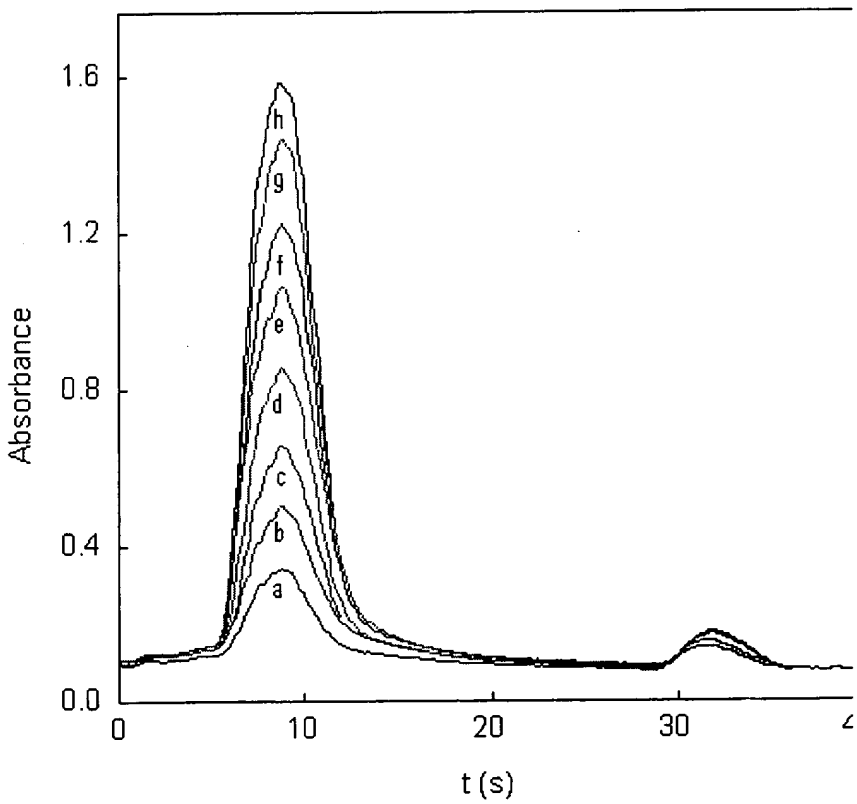
Timing sequence of the analyser

**(BT) back,
(LWT) load-wash,
(FT) forward and
(ST) stop time.
(SSV) Stream selection valve.
Bars indicate pump (P) and injection valve (IV) functions.
h_1 is the sample blank and h_2 the measured signal.**



Peaks acquired during peroxide determination

**Standards of
(a) 5.0, (b) 15.0,
(c) 25.0, (d) 35.0,
(e) 50.0, (f) 60.0,
(g) 70.0 and (h) 80.0
peroxide value (meq O_2/kg oil).**



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Questions and comments to author: Dr C.A. Georgiou, cag@aua.gr
Phone: +3010-5294248, fax: +3010-5294265
Chemistry Laboratory, Agricultural University of Athens, Greece